0273-2289/93/3940—0107$03.20

Anaerobic Bioconversion of Municipal Solid Wastes

Effects of Total Solids Levels on Microbial Numbers and Hydrolytic Enzyme Activities

C. J. RIVARD,* N. J. NAGLE, W. S. ADNEY, AND M. E. HIMMEL

Applied Biological Sciences Branch, Alternative Fuels Division, National Renewable Energy Laboratory, Golden, CO 80401

ABSTRACT

The anaerobic bioconversion of municipal solid wastes (MSW) produces both a valuable fuel product (methane) and a residue useful as a soil amendment. The application of high-solids fermentation technology offers improved economics over the more traditioinal low-solids fermentation systems. An important benefit of the high-solids process is the reduction in process water, which results in smaller fermentation reactors, and thus lower capital and operating costs. However, the anaerobic bioconversion process appears to be more efficient at high-solids as compared to low-solids levels. To understand the effects of solids levels on the anaerobic bioconversion process more thoroughly, representative high-solilds and low-solids anaerobic reactor systems processing identical MSW feedstocks are compared with respect to fermentation performance, total microbial cell number, and important hydrolytic enzyme activities.

Index Entries: Anaerobic digestion; MSW; solids, hydrolytic enzyme, microbial numbers.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Anaerobic digestion is a biological process that converts the biodegradable organic fraction of feedstocks, such as municipal solid waste (MSW), to an energy product (methane) and a nitrogen/mineral-rich soil enhancer. If the process is to be effective in the disposal of organic wastes, it should reduce the mass of the material, reduce or eliminate further biological decomposition potential, and preferably result in no waste streams itself (such as the production of byproducts [compost]). However, the composition of the organic fraction of MSW is complex because of the inherent heterogeneity of this waste stream, as well as variations resulting from geographical location and season (*1,2*). Of the various simple and complex components in MSW feedstocks, cellulose is the major polymer. Its hydrolysis has been identified as one of the most important limiting steps in the anaerobic decomposition of MSW to methane (*3,4*). In the scientific literature, sparse information exists that describes either the cellulose contents in MSW or analyzes the level of cellulose breakdown in anaerobic digestion systems treating MSW (*5,6*). A more routine estimation of bioconversion efficiency may be obtained through analysis of volatile solids destruction of the added feedstocks. However, in at least a few studies, the breakdown of the cellulose component of MSW feedstocks was found to require extensive contact times (retention times) in order to achieve substantial bioconversion without enzymatic supplementation. Aerobic biological systems require retention times of 21–28 d (*7,8*), and anaerobic systems require 20–30 d (*4,8,9*) for substantial cellulose bioconversion.

Improved volatile solids degradation rates, and in particular increased rates of cellulose hyrolysis, have been achieved by using specialized consortia from the rumen (*10*) or by using fungal-derived enzyme systems (*11*) for MSW feedstocks. However, baseline data for hydrolytic enzyme activities (especially cellulase activities) and viable microbial populations in anaerobic digestion systems processing an MSW feedstock have not been demonstrated to a large extent in the scientific literature.

In order to evaluate the level of cellulase enzyme activity resident in anaerobic digester systems, assay protocols have recently been developed that measure cellulase enzymes accurately and reproducibly (*12*). Enzymatic analyses of digester sludge samples have demonstrated that the supernatant contains no appreciable levels of hydrolytic enzymes. Rather, resident hydrolytic enzyme activities were found to be associated almost exclusively with the particulate fraction of digester sludge (*12*). Additionally, specific enzyme activities were found to correlate well with conventional methods for determining cellulose removal (i.e., acid detergent fiber analysis) in seven different reactors (*13*).

This study serves to evaluate and compare in a preliminary manner the effects of anaerobic digestion solids levels on the relative, viable microbial cell numbers and resident cellulolytic enzyme activities. Both

low-solids continuous-stirred tank reactors (CSTR) and novel high-solids reactor systems fed a high-cellulose MSW feedstock are utilized.

MATERIALS AND METHODS

Feedstocks

The MSW feedstock used in this study was obtained from Future Fuels, Inc., Thief River Falls, MN. The MSW was processed using a combination of mechanical and manual separation. The MSW feedstock was obtained in two fractions, which included the food/yard waste fraction, as well as the paper and paperboard materials (also referred to as refuse-derived fuel [RDF] in the form of densified pellets). The food/yard waste fraction was stored at 4°C until it was blended with the RDF-MSW fraction. The food/yard waste was screened using a 3/4-in tray sieve, and plastic materials were removed by hand. The RDF-MSW was size reduced from the storage pellets using a knife mill (All Steel, Inc., Brunswick, NJ) equipped with a 3/8-in round hole rejection screen. These materials were weighed separately, added to a large-scale cube blender at 180 lb total weight (50–50% mix), and blended with 40, 5-in ceramic balls for approx 48 h. The mixed MSW was again screened using the 3/4-in tray sieve before it was packaged into plastic drum liners for storage. Most of the mixed MSW was stored at −20°C until use. The mixed MSW feedstock was evaluated following mixing, and determined to be 72.7% total solids (TS), 12.5% ash, and 87.5% volatile solids (VS, as a percentage of the TS).

Previous investigations of the anaerobic bioconversion of MSW feedstocks identified the need for nutrient supplementation (*14*). Therefore, a nutrient solution as previously described (*14*) was added to adjust the moisture content of the feedstock, as well as to ensure sufficient nutrients for robust biological activity.

Biochemical Methane Potential (BMP) Analysis

The BMP assays were performed as previously described (*15*) to determine the ultimate yields of conversion of the feedstocks by the anaerobic consortium. Studies were conducted in 155-mL serum bottles at 37°C and mixed using an orbital shaker. Biogas production was measured using a pressure transducer equipped with a 22-gage needle for penetration into and subsequent overpressure release from the serum bottle.

Low-Solids Digester Operation

Four anaerobic digesters with 3.5-L working volumes and semicontinuous stirring (15 min of each 1/2 h) were constructed and operated as previously described (*16,17*). The digesters were maintained in a 37°C

constant temperature warm room. The anaerobic reactors were batch fed daily a volume of MSW plus nutrient supplement slurry to maintain a 14-d retention time. In the batch-feeding protocol, a volume of effluent equivalent to the volume of feed added was removed daily to maintain the reactor sludge volume at 3.5 L. In the operation of the reactors, the solids retention time was equivalent to the hydraulic retention time.

High-Solids Digester Operation

The laboratory-scale high-solids reactors used in this preliminary study were described previously (*18*), and consist of a cylindrical glass vessel positioned with a horizontal axis, capped at each end. The agitator shaft runs horizontally along the axis of the cylinder. Mixing is obtained with a rod-type agitator (tines) attached to the shaft at 90°C angles and in opposing orientation. Shaft rotation is provided by a low-speed, high-torque, hydraulic motor (Staffa, Inc., England). The glass vessel was modified with several ports, including two 3/4-in ports for liquid introduction and gas removal and a 2-in ball valve (Harrington Plastics, Denver, CO) used for dry feed introduction and effluent removal.

The four high-solids reactors used in this study were maintained at 37°C in a temperature-controlled warm room. The reactors were batch fed daily by adding the relatively dry MSW feedstock and a liquid nutrient solution. Sludge was removed from the reactor on a biweekly basis and stored at 4°C until it was analyzed.

Feedstock/Digester Effluent Analysis

The solids concentrations of both the feedstock and digester effluent samples were determined using 1-g aluminum weight tins. A 20- to 30-g sample was loaded into preweighed tins and dried for 48 h at 45–50°C. The dried sample was then cooled to room temperature in a laboratory desiccator and weighed using a Sartorius balance (Model 1684MB). The percent TS was calculated on a weight/weight basis, and the percents VS and ash were determined by combustion of the dried samples at 550°C for 3 h in a laboratory-scale furnace.

Feedstock materials were analyzed for levels of carbon oxygen demand (COD) as previously described (*19*). The COD assay employed the micro-determination method with commercially available "twist tube" assay-vials (Bioscience, Inc., Bethlehem, PA).

Levels of volatile organic acids (C_2-C_5 iso- and normal acids) were determined by gas–liquid chromatography (GLC). A Hewlett-Packard Model 5840A gas chromatograph equipped with a flame ionization detector, a Model 7672A autosampler, and a Model 5840A integrator (all from Hewlett-Packard) were used. The chromatograph was equipped with a glass column packed with Supelco 60/80, Carbopack C/0.3%, Carbowax 20M/0.1% H_3PO_4 for separations. The feedstock was also analyzed with

respect to specific polymer content as determined by the standard forage fiber analyses of acid detergent fiber (ADF) and neutral detergent fiber (NDF) as previously described (*20*).

Gas Analysis

Total biogas production in high-solids reactor systems was measured daily using precalibrated wet tip gas meters (Rebel Point Wet Tip Gas Meter Co., Nashville, TN). Total biogas production in low-solids CSTR systems was determined from calibrated water-displacement reservoirs. The composition of the biogas produced was determined by gas chromatography as previously described (*21*). For this analysis, a Gow-Mac (Model 550) gas chromatograph equipped with a Porapak Q column and a thermal conductivity detector with integrating recorder was used.

Theoretical Methane Yield

The theoretical methane yield for the MSW feedstock was calculated as previously described (*15*) from the feedstock COD value. The ratio of actual methane yield for a given anaerobic fermentation system to the theoretical methane yield calculated from the feedstock COD value is a direct reflection of the organic carbon conversion of the substrate added.

Cellulase Enzyme Assay Methodology

Digester samples from both low-solids and high-solids systems fed the MSW feedstock were sampled on a weekly basis over a 4-wk period for analysis. Digester sludge samples were first dilued to 1–2% TS. The diluted samples were then split into two equal 30-mL samples. One of the samples was used for analysis of total solids content. The second sample was used for enzyme assessment. For enzymatic assays, the diluted digester sludge sample was subjected to centrifugation at 15,000 $\times g$ for 20 min at room temperature to concentrate the particulate fraction. The supernatant was discarded, and the pellet was resuspended in 15 mL of 100 m*M* Tris buffer at pH 7.0. Sodium dodecyl sulfate (SDS) was then added to obtain a final concentration of 0.1%, and the sample was gently mixed for 16–20 h. The samples were then centrifuged at 15,000 $\times g$ for 20 min at room temperature. The supernatant was removed and filtered using a 0.45-μm disposable Acrodisk syringe filter (Gelman Sciences, Ann Arbor, MI). The filtered supernatant was then assayed for various cellulase enzyme activities. As a control, the supernatant from the initial particulate concentration was assayed for enzyme activity, and no activity above the assay background was determined.

The determination of β-D-glucosidase (EC 3.2.1.21), endoglucanase (EC 3.2.1.4), and exoglucanase (EC 3.2.1.74) in detergent extracts of digester sludge samples was performed as previously described (*13*). One

modification to the assay protocol for exoglucanase activity was the substitution of phosphate-swollen cellulose in 100 m*M* Tris, pH 7.0, instead of Whatman #1 filter paper strips.

Microbial Enumeration

As a measure of the total viable microbial numbers from sludge samples of both high- and low-solids reactor systems, a rich plating medium was utilized to obtain growth of a wide spectrum of microorganisms. The growth medium contained the following components per liter of distilled water: K_2HPO_4, 0.7 g; KH_2PO_4, 0.54 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; $CaCL_2 \cdot 2H_2O$, 0.2 g; NH_4Cl, 0.5 g; yeast extract (difco), 7.5 g; bacto-peptone (difco), 7.5 g; glucose, 5.0 g; trace vitamin solution (22), 10 mL; trace mineral solution (22), 10 mL; cysteine HCl, 0.3 g; resazurin, 0.001 g; and agar, 15.0 g. The medium components were prepared under anaerobic conditions as previously described (23) using 500-mL serum bottles. Following sterilization of the agar growth medium, the agar was tempered in a 60°C water bath. The tempered-agar medium was transferred to an anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, MI). Sterile disposable Petri dishes were previously transferred to the chamber to allow outgassing of oxygen from the plates. The agar medium was transferred to Petri dishes and allowed to solidify. Poured plates were maintained within the chamber for 5 d to reduce moisture in advance of spread plating. Representative digester sludge samples were serially diluted in 9-mL blanks containing 50 m*M* K_2HPO_4 buffer (pH 7.4). During dilution, the sludge samples were repetitively vortexed to dislodge microbial cells that may be associated with the particulate fraction of the sample. A 0.1-mL aliquot of each dilution within the series was pipeted onto individual plates and spread plated. The inoculated plates were allowed to incubate for 5 d within the chamber at room temperature (20°C). Following incubation, the plates were removed from the chamber, and colonies were counted using a dark-field counter.

RESULTS

Initially, the anaerobic bioconversion of the processed MSW feedstock was evaluated using the BMP method. Because an anaerobic microbial consortium had previously been adapted to the bioconversion of this waste, the fermentation in BMP assays was rapid; most of the fermentation was complete after 10 d. The methane yield from the BMP assay following an extensive incubation period of 90 d (BMP_{90}) was 258 ± 16 mL CH_4/g of VS added. The yield from a BMP_{90} assay may be considered as an indication of the ultimate biodegradable fraction of the waste. When the BMP_{90} yield is compared to the theoretical yield based on the COD content of the MSW feedstock, the level of bioconversion is 64.7%.

Table 1
Comparison of Anaerobic Bioconversion Performance for Low- and High-Solids Digestion Systems

	Organic loading rate, OLR		Biogas productivity	Methane content	Methane yield	% Bioconversion	
Digester	g VS/L·d	g COD/L·d	mL/L·d	%	mL/L·d	COD	BMP_{90}
Low solids	2.0	2.3	918±129	59.8	549±77	68.2	106.4
High solids	14.0	15.3	6225±880	60.1	3741±529	69.9	103.6

Table 2
Comparison of Anaerobic Bioconversion Fermentation Parameters for Low- and High-Solids Digestion Systems

	Total solids, %					
Digester	Feed	Effluent	pH	Volatile fatty acids[a]	Total solids reduction	Bulk reduction
Low solids	7.3	4.9±0.3	7.4±0.2	< 10 m*M*	34%	2.5%
High solids	30.0	21.5±0.3	7.7±0.1	< 10 m*M*	35%	10.5%

[a]Volatile fatty acids are cumulative for C-2 to C-5 iso- and normal acids.

The anaerobic bioconversion of the MSW feedstock was evaluated for more continuous systems (daily batch fed) using both conventional low-solids digester technologies, i.e., semicontinuous stirred tank reactors and a novel high-solids reactor technology. The results, as shown in Tables 1 and 2, indicate a stable fermentation for both low-solids and high-solids reactor systems as indicated by the fermentation pH and low cumulative volatile fatty acid concentration in the sludge. The organic loading rate was selected for each system based on obtaining a robust fermentation without undue stress imposed by an excessive organic loading. The methane product yields were comparable for both low- and high-solids systems with respect to the theoretical yield (COD), as well as the ultimate yields (BMP_{90}). The methane yields in both systems were slightly greater than those determined in the BMP_{90} assay, which is consistent with our previous research results for a variety of feedstocks. Although the digester solids level did not affect the TS reduction, the effect on bulk reduction is significant because of the level of process water in the low-solids digester system, which is unchanged during the fermentation.

Both low- and high-solids digestion systems were also evaluated with respect to resident cellulase enzyme activities using a series of detergent extractions developed by Adney et al. (*12*). These extraction procedures, which utilize low detergent concentrations, were used to extract active enzymes from the particulate fraction of sludge samples taken from both

Table 3
Comparison of Hydrolytic Enzyme Levels
for Low- and High-Solids Digestion Systems

Cellulase activity	Low solids	High solids
β-D-glucosidase (U/min)		
U/mL digester sludge	0.012±0.002	0.073±0056
U/g digester sludge solids	0.24±0.04	0.34±0.26
U/g volatile solids added·d	6.0±1.0	5.2±4.0
Endoglucanase (μmol glucose released/min)		
Activity Units/mL digester sludge	0.016±0.006	0.239±0.039
Activity Units/g digester sludge solids	0.33±0.12	1.11±0.18
Activity Units/g volatile solids added·d	8.0±3.0	17.1±2.8
Exoglucanase (μmol glucose released/min)		
Activity Units/mL digester sludge	0.014±0.002	0.058±0.002
Activity Units/g digester sludge solids	0.29±0.04	0.27±0.01
Activity Units/g volatile solids added·d	7.0±1.0	4.1±0.1

Table 4
Comparison of Microbial Enumerations
for Low- and High-Solids Digestion Systems

Total cell number, Colony-Forming Units, CFU	Low solids	High solids
CFU/mL sludge	$4.7\pm1.1\times10^{7}$	$3.6\pm0.7\times10^{7}$
CFU/g digester sludge solids	$9.6\pm2.2\times10^{8}$	$1.7\pm0.3\times10^{8}$
CFU/g volatile solids added/d	$2.4\pm0.5\times10^{10}$	$2.6\pm0.5\times10^{9}$

reactor systems. Table 3 describes the results of individual enzymatic assays for β-D-glucosidase, endoglucanase, and "apparent" exoglucanase. (The term "apparent" is used here in referring to the exoglucanase activity, because phosphate-swollen cellulose was substituted as the substrate in this assay in place of Whatman #1 filter paper strips.) Enzymatic activity expressed in activity units per gram of digester sludge solids is comparable for low-solids and high-solids systems with the exception of endoglucanase activities, which are threefold greater in the high-solids system. However, the level of enzymatic activity are significantly higher in the high-solids system when evaluated on a volume basis (i.e., per milliliter) because of the greater level of solids in this system. When comparing the resident hydrolytic enzyme activities with the system organic loading rate, the levels are also comparable.

The analysis of total microbial numbers from digester sludge from low-solids and high-solids systems was evaluated using a rich enumeration medium. The data as shown in Table 4 indicate slightly higher (although similar) microbial numbers for low-solids digesters as compared to the

high-solids system based on a per milliliter basis. When the total cell numbers are compared based on digester sludge solids, the data indicate a substantially higher number for the low-solids digester system. Likewise, when the total microbial numbers are based on the system organic loading rate, nearly a tenfold increase in total cell number is evident for the low-solids digester system.

DISCUSSION

The processes MSW feedstock obtained from Thief River Falls, MN, represents a mixture of residental and industrial wastes generated from an average small municipality. In using this waste material, a significant portion of the inert materials was removed and recycled, which maximized the biodegradable portion for the ensuing biological disposal system. Although this waste material was relatively well sorted, the level of anaerobically biodegradable organics constituted approx 65% as determined by the BMP_{90} assay, i.e., when compared to the COD content of the MSW feedstock. It is important to note that the BMP_{90} data represent the bioconversion that is possible with an infinite incubation period (i.e., 90 d compared to digestion systems with retention times of 14–28 d).

The levels of conversion determined for both the low-solids and high-solids digestion systems were comparable and represented essentially 100% of the conversion determined in the BMP_{90} assay. Although the TS reduction for the two systems was substantial (34–35%), the overall bulk reducton of the feedstock was notably lower, especially in the low-solids digestion system because of the high level of process water.

The level of cellulase enzyme activity was comparable for both systems, based on grams of sludge solids. However, because the solids content was greater in the high-solids digestion system, the cellulase activity per milliliter of sludge was substantially greater. The hydrolytic power of both low-solids and high-solids systems was similar, as shown by the levels of cellulase enzyme activities per gram of VS fed to each system per day.

The relative number of total microbial cells, determined from cultivation on rich medium, indicated that the high-solids system was on the order of five- to sixfold lower in cell number per gram of digester sludge solids when compared to the low-solids digestion system. In fact, evaluation of the total microbial numbers, which also serves as a measure of biodegradative power of the system, compared to the system organic loading, indicated that the low-solids digestion system was on the order of nine times greater than the high-solids system.

In general, both the low-solids and high-solids systems were effective in bioconversion of the processed MSW feedstock. The level of bulk reduction in the high-solids system was substantially greater than that of

the low-solids system because of the higher level of unreacted water in the low-solids system. Additionally, the potential for a greater bulk reduction of the feedstock would exist if the high-solids process were operated at a solids level closer to the maximum solids level for the system (approx 35–40%). The hydrolytic enzymatic power relating to cellulose breakdown of each system was comparable on a sludge solid basis. However, because the high-solids digestion system contained a greater level of solids, and thus enzyme activity, the level of enzymatic activity per gram of VS fed was similar even though the high-solids digestion system was fed at seven times the organic loading rate of the low-solids digester. Analysis of the ratio of the total microbial numbers to sludge solids indicated a greater level of microbial biocatalysts in the low-solids digestion system. Although this result was unexpected in light of the level of cellulase enzymes present in both systems, it is important to note that the proportion of hydrolytic microorganisms in the total microbial number remains unknown.

ACKNOWLEDGMENT

This work was funded by the Waste Management Program of the US Department of Energy.

REFERENCES

1. Schwartz, S. C. and Brunner, C. R. (1983), *Energy and Resource Recovery from Waste*, Noyes Data Corp., New Jersey.
2. US Environmental Protection Agency (1974), *Third Report to Congress*, Pub. No. SW-161, Washington, D.C.
3. Boone, D. R. (1982), *Appl. Environ. Microbiol.* **43,** 57.
4. Noike, T., Endo, G., Chang, J.-E., Yaguchi, J.-I., Matsumoto, J.-I. (1985), *Biotechnol. Bioeng.* **27,** 1482.
5. Biljetina, R., Srivastava, V. J., Fannin, K. F., Janulis, J. A., and Henry, M. P. (1987), *Annual Report for Walt Disney Imagineering, Contract No. 9MP-86-1.*
6. Stenstrom, M. K., Ng, A. S., Bhunia, P. K., and Abramson S. D. (1983), *J. Environ. Eng.* **109,** 1148.
7. Verachtert, H., Ramasamy, K., Meyers, M., and Bevers, J. (1982), *J. Appl. Bacteriol.* **52,** 185.
8. Vinzant, T. B., Adney, W. S., Grohmann, K., and Rivard, C. J. (1990), *Appl. Biochem. Biotech.* **24/25,** 765.
9. Gujer, W. and Zehnder, A. J. B. (1983), *Wat. Sci. Tech.* **15,** 127.
10. Gijzen, H. J., Zwart, K. B., Teunissen, M. J., and Vogels, G. D. (1988), *Biotech. Bioeng.* **32,** 749.
11. Clanet, M. and Durand H. (1988), *Biotech. Bioeng.* **32,** 930.
12. Adney, W. S., Rivard, C. J., Grohmann, K., and Himmel, M. E. (1989), *Biotech. Apl. Biochem.* **11,** 387.

13. Rivard, C. J., Adney, W. S., and Himmel, M. E. (1991), *Enzymes in Biomass Conversion*, Leatham, G. and Himmel, M. E., eds., American Chemical Society Books, Washington, D.C.
14. Rivard, C. J., Vinzant, T. B., Adney, W. S., Grohmann, K., and Himmel, M. E. (1990), *Biomass* **23,** 201.
15. Owen, W. F., Stuckey, D. C., Healy, J. B., Young, L. Y., and McCarty, P. L. (1979), *Water Res.* **13,** 485.
16. Rivard, C. J., Bordeaux, F. M., Henson, J. M., and Smith, P. H. (1987), *Appl. Biochem. and Biotech.* **17,** 245.
17. Henson, J. M., Bordeaux, F. M., Rivard, C. J., and Smith, P. H. (1986), *Appl. Environ. Microbiol.* **51,** 288.
18. Rivard, C. J., Himmel, M. E., Vinzant, T. B., Adney, W. S., Wyman, C. E., and Grohmann, K. (1989), *Appl. Biochem. and Biotech.* **20/21,** 461.
19. Greenberg, A. E., Conners, J. J., and Jenkins, D., eds. (1981), in *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, D.C.
20. Goering, H. K. and Van Soest, P. J. (1970), *US Dept. of Agriculture Handbook #379.*
21. Rivard, C. J., Himmel, M. E., and Grohmann, K. (1985), *Biotech. Bioeng. Symp.* **15,** 375.
22. Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R., and Wolfe, R. S. (1979), *Microbiol. Rev.* **43,** 260.
23. Hungate, R. E. (1969), in *Methods in Microbiology*, Norris, J. R., and Ribbons, D. W., eds., Academic, New York.